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TRANSCRIPTION OF T CELL ANTIGEN RECEPTOR GENES IS INDUCED BY PROTEIN KINASE C ACTIVATION[1]

TULLIA LINDSTEN,[2]* CARL H. JUNE,† AND CRAIG B. THOMPSON*

*From the * Howard Hughes Medical Institute and Departments of Medicine and Microbiology/Immunology, University of Michigan, Ann Arbor, MI 48109, and † Naval Medical Research Institute, Bethesda, MD 20814*

The regulation of expression of the TCR-α and -β genes was studied in the human T cell tumor line Jurkat. Treatment of the cells with PMA was shown to decrease the surface expression of the TCR-α/β/CD3 complex. Subsequent to PMA-induced modulation of the TCR/CD3 complex, increases in the mRNA levels of both the TCR-α and -β genes were observed reaching a maximum 12 h after stimulation. Other T cell activators were also examined for their ability to increase TCR-α and -β mRNA expression. Only agents that activate protein kinase C were shown to induce expression of the TCR-α and -β genes. The observed increases in TCR-α and -β gene mRNA levels were not the result of a uniquely derived Jurkat subline. Similar inductions of TCR-α and -β mRNA levels were observed in an independently maintained Jurkat cell line. In both cell lines, elevations of TCR gene expression was accompanied by a decline in the expression of the c-*myc* protooncogene. PMA induction of TCR-α and -β mRNA was shown to occur in the presence of the protein synthesis inhibitor cycloheximide. The 1.6-kb TCR-α and the 1.0-kb $D_\beta J_\beta C_\beta$ TCR-β gene transcripts were fully induced in the presence of cycloheximide, whereas the 1.3-kb $V_\beta D_\beta J_\beta C_\beta$ transcript was only partly induced in the presence of cycloheximide. Run-on transcription assays demonstrated that the increase in TCR-α and -β mRNA levels could be entirely accounted for by increases in the transcription rate of both genes after PMA induction. Thus, in summary, protein kinase C stimulation leads to TCR-α/β modulation in Jurkat cells and an increase in steady state TCR-α and -β mRNA levels as a result of transcriptional activation of both genes.

The α/β TCR is a disulfide-bonded heterodimer consisting of an acidic α- and a basic β-chain (1–3). The α- and β-chains are encoded by separate genes that are assembled during T cell development by rearrangement of V, J, C, and in the case of the β-chain, D region gene segments (4–8). The TCR is associated on the cell surface with the CD3 complex of proteins, which consists of two glycoproteins, γ and δ and two nonglycosylated proteins, ϵ and ζ (9–14). During activation of T cells with Ag, mitogens or PMA, serine phosphorylation of the γ-chain takes place (15–17). In addition, the ζ-chain is phosphorylated on tyrosine residues after Ag and mitogen but not PMA activation (16, 18). Activation of T cells by either antibodies directed against the TCR/CD3 complex or PMA leads to a rapid decrease in cell surface expression of the TCR/CD3 complex (3, 17, 19–21). Although some workers have reported that the TCR/CD3 complex can be shed from the cell surface (19), several recent studies suggest that the decrease in the surface expression of the TCR/CD3 complex after PMA or anti-CD3 stimulation is due primarily to internalization (22, 23). In view of the reported modulation of the TCR/CD3 complex, we have investigated TCR-α and TCR-β gene transcription and steady state mRNA expression after phorbol ester-mediated activation of the human T cell tumor line Jurkat. The Jurkat cell line was chosen as a model because of the availability of cDNA probes specific for Jurkat TCR-α and TCR-β genes (24, 25) as well as clonotypic antibodies directed against the Jurkat Ag receptor (26).

We confirmed that, in the Jurkat cell line, PMA treatment leads to a rapid decrease in TCR/CD3 surface expression apparent within 1 h after addition of PMA. In contrast, the steady state mRNA levels of both TCR α- and TCR β-chain genes do not begin to increase until several hours after stimulation with either PHA or PMA of Jurkat cells. Within 6 to 12 h after stimulation both TCR-α and TCR-β steady state mRNA levels increase 5- to 10-fold. Both the 1.3 kb TCR-β mRNA, shown previously to represent full-length $V_\beta D_\beta J_\beta C_\beta$ transcripts, and the 1.0-kb TCR-β mRNA, which represents sterile $D_\beta J_\beta C_\beta$ transcripts (24, 27), are inducible. These increases in expression are due to corresponding increases in transcription as assayed by nuclear run-on experiments. Furthermore, the increased expression of the 1.6-kb TCR-α mRNA and the 1.0-kb $D_\alpha J_\alpha C_\alpha$ mRNA are independent of new protein synthesis, whereas the increase of the functional 1.3-kb $V_\alpha D_\alpha J_\alpha C_\alpha$ mRNA is partially dependent on new protein synthesis. These results suggest that mechanisms for both coordinate and independent regulation of the TCR-α and TCR-β genes exist. Our data also suggest the presence of an inducible regulatory element in the vicinity of the C_α region, possibly an enhancer sequence.

MATERIALS AND METHODS

Cells. Two independently maintained Jurkat T cell tumor cell lines were used in these studies. Jurkat E (Jurkat strain FHCRC [29])

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[2] T. L. is a Leukemia Society of America Special Fellow. Please address all correspondence and requests for reprints to Dr. T. Lindsten, Howard Hughes Medical Institute, 1150 W. Medical Center Dr., MSRB I, Room 4510, Ann Arbor, MI 48109.

and Jurkat II (the generous gift of Dr. J. M. Leiden, Howard Hughes Medical Institute, University of Michigan, MI). Unless otherwise indicated, the experiments shown in this paper used the Jurkat I cell line. Exponentially growing Jurkat cells maintained in RPMI with 10% FCS were stimulated with the following agents: PHA (Sigma Chemical Co., St. Louis, MO) at 10 μg/ml, PMA (Sigma) at 3 ng/ml or 10 ng/ml, or mAb 9.3 (anti-CD28) produced as previously described (29) and used at a final concentration of 100 ng/ml. In some experiments, the cells were treated with CHX[3] (Sigma) at 10 μg/ml.

Northern blot analysis. Cells were harvested and total cellular RNA was prepared using guanidinium isothiocyanate, as previously described (30). The samples were equalized for ribosomal RNA by ethidium bromide staining after separation on 1% non-denaturing agarose gels, as described (31). The equalized RNA samples were then separated on 1% agarose-formaldehyde gels and transferred to nitrocellulose. Hybridization was in 50% formamide, 10% dextran sulfate, 5× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate), 1× Denhardt's solution, 25 mM sodium phosphate, pH 6.5, and 250 μg/ml *Torula* yeast RNA, at 42°C for 16 to 20 h. After hybridization, filters were washed twice for 5 min in 1× SSC, 0.1% SDS at 20°C and then twice for 30 min in 0.1× SSC, 0.1% SDS at 55°C.

DNA probes. The TCR-α cDNA clone pY1.4 contains a full length copy of the TCR-α gene (25), and the TCR-β cDNA probe Jurkat-β2 contains J and C regions of the TCR-β gene (24). Both probes were obtained from a Jurkat cDNA library (24, 25). β-Actin and GPD cDNA probes have been previously described (32, 33). The 28S ribosomal gene probe was prepared by digesting the rA genomic clone (34) with *Eco*RI and *Bam*HI to generate a 1.6-kb fragment, which was subcloned into pGEM4. Inserts (50 to 100 ng) of the above mentioned plasmids, obtained after digestion with the appropriate restriction endonucleases and separation on low melting point agarose, were labeled by nick translation to a sp. act. of 3×10^8 to 9×10^8 cpm/μg. Labeled probes were added to a final concentration of 10^6 cpm/ml of hybridization mix.

Run-on transcription assays. Nuclei were prepared as previously described (35) and resuspended to 50 mM Tris, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, and 0.1 mM EDTA. Nuclear run-on transcription assays were performed using fresh nuclei as described by Groudine et al. (35) and modified elsewhere (36). Generally, 3×10^7 to 4×10^7 cpm/5×10^7 cells were obtained. In a given experiment, identical cpm ere hybridized at a final concentration of 10^7 cpm/ml to Southern ots of 5 μg of plasmids on which inserts had been separated from vector by using appropriate restriction endonucleases. Hybridization and washing conditions have been described elsewhere (36).

Quantitation of cell surface expression. mAb FITC-conjugated anti-Leu-4 (CD3), FITC-conjugated anti-Leu-5 (CD2), and biotin-conjugated WT31 (an antibody directed against the "framework" region of the Jurkat α/β TCR) were obtained from Becton Dickinson (Mountain View, CA.). Anti-clonotypic TCR β-chain mAb C305, kindly provided by Dr. A. Weiss, Howard Hughes Medical Institute, University of California San Fransisco, CA was previously described (26). Cells were incubated with saturating amounts of mAb and, when necessary, stained with the second-step reagent FITC-conjugated goat-anti-mouse Ig (Kirkegaard and Perry Laboratories, Gaithersburg, MD) or FITC-avidin (Dako Corp., Santa Barbara, CA). Cells were then washed and fixed with 2% paraformaldehyde. Flow cytometry was performed on an Ortho cytofluorograph model 50HH (Westwood, MA) using forward and right angle light scatter to discriminate debris, and fluorescence was quantitated using a linear scale.

RESULTS

PMA treatment decreases the surface expression of the TCR/CD3 complex. Before embarking on the mRNA expression and transcription studies, we wanted to confirm previous studies showing that only 1 h of PMA treatment of both normal T cells and tumor cell lines leads to a decrease in cell surface expression of the TCR/CD3 complex (17, 20, 21). Exponentially growing Jurkat cells were treated with PMA for 1 or 10 h and then stained with the Jurkat C305 clonotypic anti-TCR antibody, the WT31 framework anti-TCR antibody, and an anti-CD3 antibody. Table 1 shows that at 1 h there is approximately a 60% decrease in mean fluorescence intensity staining with anti-CD3 and the two anti-TCR antibodies,

TABLE 1

PMA decreases surface expression of TCR/CD3

	Time (h)	Mean Fluorescence Intensity[a]			
		CD3	TCR (C305)	TCR (WT31)	CD2
Medium	1	148	274	103	478
PMA	1	54	110	57	583
Medium	10	155	313	94	487
PMA	10	29	77	6	595

[a] Mean fluorescence intensity was quantitated on a linear scale and channel number is shown. Cells were stained with antibodies directed against CD3, CD2, and TCR as described in *Materials and Methods*.

and at 10 h the decrease is even more pronounced, reaching more than 80%. Staining with a control antibody, anti-CD2, showed a marginal increase upon PMA stimulation.

PMA treatment increases steady state mRNA levels of the TCR-α and TCR-β genes. Exponentially growing Jurkat cells were treated for 12 h with PMA and cytoplasmic RNA was prepared. The RNA samples were equalized to ribosomal RNA (see *upper panel* of Fig. 1) to provide a stable reference to compare mRNA levels of specific genes after cellular activation by the different stimuli used (31). The Northern blot in Figure 1 shows that TCR-α gene expression increased 5- to 10-fold upon stimulation of the cells with PMA for 12 h. A similar increase was seen when the Jurkat cells were treated with PMA for 12 h but not when stimulated with the mAb 9.3, which stimulates T cell activation via a pathway independent of protein kinase C, mediated by CD28 (37). Stimulation with the mAb 9.3 was used as a control showing that binding to any T cell surface structure did not induce TCR-α gene expression. Stimulation with the mAb 9.3 in addition to PMA had no further effect on the induction of TCR-α gene expression by PMA, as shown by Northern blot analysis (Fig. 1). It should be pointed out that mAb 9.3 plus PMA did not induce IL-2 production in this Jurkat subline. The TCR-β gene expression also increased with the same stimuli that enhance TCR-α gene expression. These stimuli share the property of being activators of protein kinase C (38). Interestingly, both the full length 1.3 kb $V_\beta D_\beta J_\beta C_\beta$ and the 1.0-kb $D_\beta J_\beta C_\beta$ transcripts were inducible. Cells harvested at 1 h after PMA stimulation showed no increase in steady state TCR-α or -β mRNA levels. Near maximal induction of transcripts from both genes was apparent by 6 h (data not shown). In contrast, the expression of the proto-oncogene c-*myc* decreased after stimulation with PHA, PMA, or mAb 9.3 plus PMA. A similar decrease in c-*myc* expression has been noted in several cell types induced to cease proliferation or undergo further differentiation (39–41).

A second Jurkat cell line (Jurkat II) was also studied, because independently maintained tumor cell lines often develop distinct characteristics. Jurkat II displayed a several-fold higher base line expression of the TCR-α gene as shown in Figure 2. The level of the TCR-β gene was similar in the two cell lines. Upon PMA stimulation, using two different concentrations (3 and 10 ng/ml) the TCR-α gene was induced to the same level in Jurkat I and II. Because of the higher base line of TCR-α gene expression in Jurkat II compared with Jurkat I, the absolute fold increase was less. The TCR-β gene was induced by the same degree and to the same level in both Jurkat I and II. No difference in induction was seen with the two

[3] Abbreviations used in this paper: CHX, cycloheximide; GPD, glyceraldehyde-3-phosphate dehydrogenase; AP-1, activation protein 1.

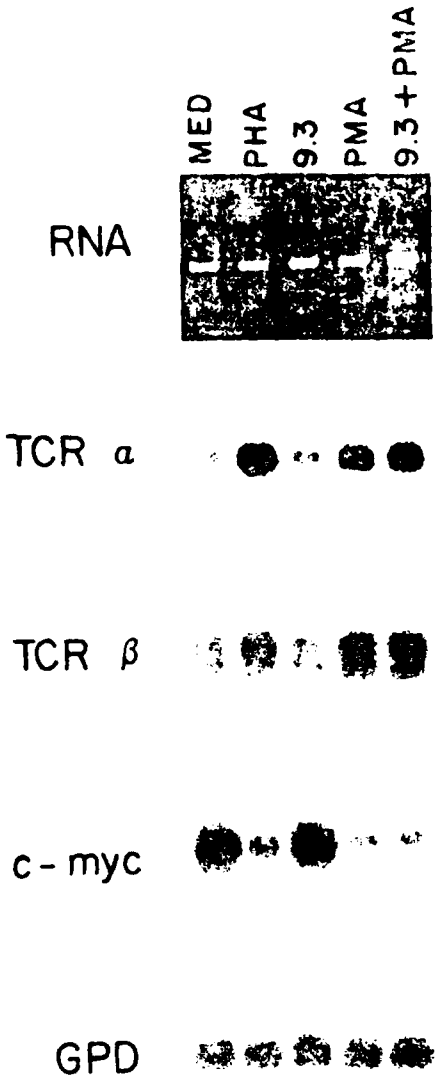

Figure 1. PMA increases TCR-α and -β steady state mRNA levels. Exponentially growing Jurkat I cells were treated for 12 h with medium (*MED*) alone, PHA, mAb 9.3, PMA, or mAb 9.3 plus PMA. RNA was extracted and equalized as described (31, 32). The *upper panel* (RNA) shows 10% of each RNA sample run on a 1% non-denaturing agarose gel, confirming the equalization. Northern blots were probed sequentially with TCR-α, TCR-β, c-*myc*, and GPD cDNA probes.

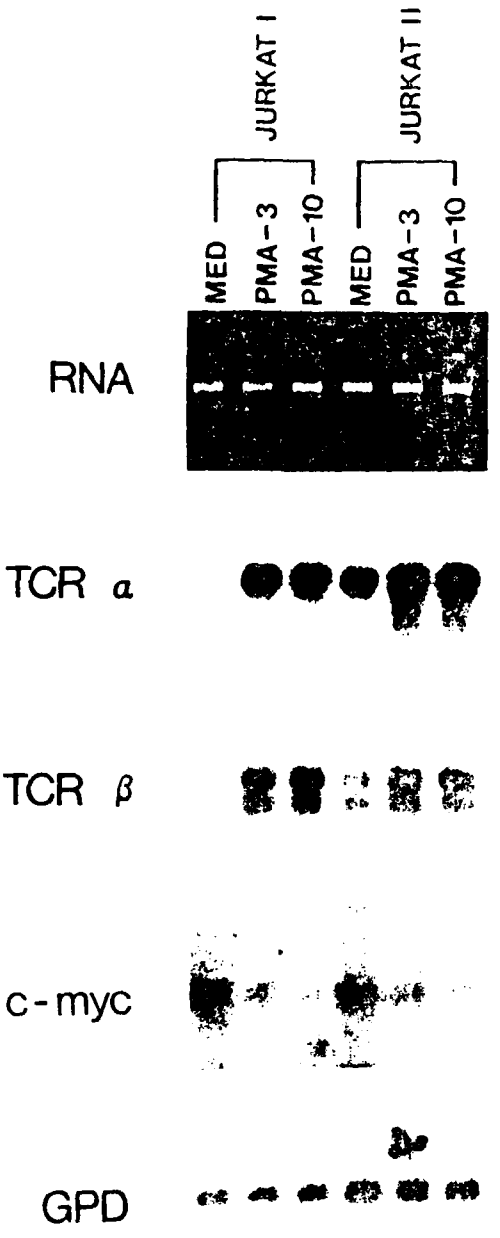

Figure 2. PMA-induced increase of TCR-α and -β steady state mRNA levels in different Jurkat cell lines. Exponentially growing Jurkat I and Jurkat II cells were treated for 12 h with medium (*MED*) alone or PMA at 3 (*PMA-3*) or 10 (*PMA-10*) ng/ml. RNA was extracted and equalized (*upper panel*) as described in legend to Figure 1. Northern blots were probed sequentially with TCR-α, TCR-β, c-*myc*, and GPD cDNA probes.

different PMA concentrations used. The expression of the household gene GPD did not vary significantly during stimulation of either cell line (Fig. 2).

The effect of cycloheximide on PMA-induced increase of TCR-α and -β mRNA levels. To determine whether PMA can induce its effects in the absence of new protein synthesis, Jurkat cells were stimulated with PMA in the presence and absence of the protein synthesis inhibitor CHX. Figure 3 shows the Northern blot analysis of this experiment. The PMA-induced increase in TCR-α gene expression is completely independent of new protein synthesis. However, the TCR-β gene expression is regulated differently. The ability of PMA to induce the 1.3-kb $V_\beta D_\beta J_\beta C_\beta$ transcript was significantly reduced in the presence of CHX whereas the PMA-induced increase of the 1.0-kb $D_\beta J_\beta C_\beta$ transcript was unaffected by CHX addition.

PMA-induced increase of TCR-α and -β steady state mRNA levels are mediated by increases in transcription. To determine whether the PMA-induced increase in TCR mRNA levels was due to increases in transcription, nuclear run-on transcription assays were performed. Nuclei were prepared from cells grown for 12 h in the presence or absence of 10 ng/ml PMA. The run-on products of these nuclei were hybridized to Southern blots of TCR-α, TCR-β, as well as control gene plasmids, where the inserts had been separated from the vector (see Fig.

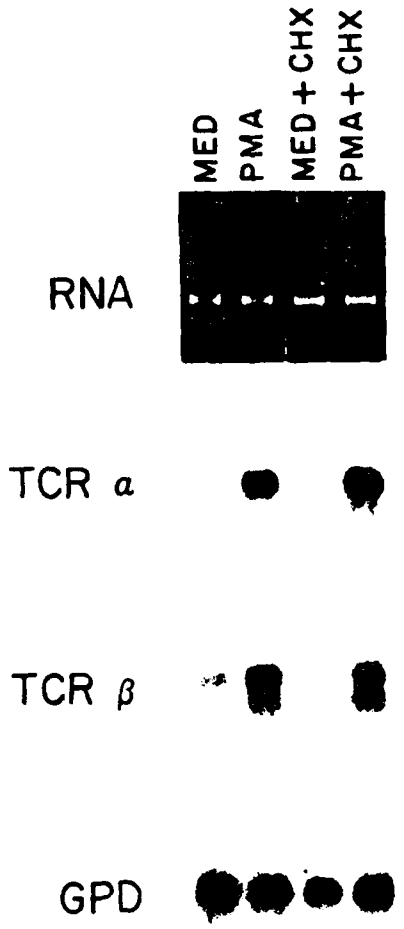

Figure 3. The effect of cycloheximide on PMA induced increase of TCR-α and -β steady state mRNA levels. Exponentially growing Jurkat I cells were treated for 12 h with either CHX alone or with CHX and PMA. RNA was extracted and equalized (see upper panel) as described in legend to Figure 1. Northern blots were probed sequentially with TCR-α, TCR-β, and GPD cDNA probes.

4*A*). Figure 4*B* shows that the base line transcription of the TCR-α and TCR-β gene in non-stimulated Jurkat cells is similar. Actin and the 28S ribosomal gene were used as control genes. No hybridization to vector (pGEM4) alone was seen. Upon stimulation with PMA, transcription of both the TCR-α and TCR-β genes were induced 5- to 10-fold as seen in the *lower panel* of Figure 4*B*. In contrast, the level of c-*myc* transcription was greatly reduced in the PMA-treated samples. Therefore, the reduction in steady-state c-*myc* RNA levels is regulated transcriptionally. Transcription of the actin and the 28S ribosomal genes did not change significantly. Run-on transcription assays of Jurkat II produced similar results. Based on these results, it appears that PMA increases the steady state mRNA levels of the TCR-α and TCR-β genes primarily by increasing their transcription rate.

DISCUSSION

In this report, we have examined the effects of PMA on the expression of the TCR-α and TCR-β genes in human Jurkat T cells. We find that, as reported for normal T cells and other T cell lines, PMA induces a rapid modulation of the TCR/CD3 complex in Jurkat cells. In addition to modulation of the TCR/CD3 complex, PMA treatment increases the steady state mRNA levels of the TCR-α and TCR-β genes. This increase in steady state mRNA levels is due to a corresponding increase in transcription. Recently, several PMA-inducible genes have been described that share a sequence motif with the ability to bind the activation protein AP-1 (43, 44). The consensus sequence of the AP-1 binding site is $^{C}_{G}$TGACT$^{C}_{A}$A, where the underlined bases have to be absolutely conserved to preserve maximal PMA inducibility (43–45). The AP-1 binding site is usually found near position −70 in the PMA-inducible genes described (43). Inspection of sequences upstream of the V regions of the TCR-α and the TCR-β genes, recently published, reveals three possible AP-1 binding sites for each gene (46, 47). For the TCR-α gene, they are located at −10 to −4 (ATGATGAA), at −30 to −24 (GTGAACCA) and at −82 to −76 (ATGAGAAA) (46). These sites all have the preserved -TGA---A sites. The same is true for the TCR β gene, where the sites are located at −52 to −46 (CTGACAGA), at −122 to −116 (CTGATTCA) and at −138 to −132 (TTGAGTTA) (47). Based on this sequence data, it is possible that one mediator of the transcriptional activation of the TCR-α and -β genes is the PMA-inducible AP-1 transcription factor. However, AP-1 binding to consensus sites upstream of the V region is unlikely to be the entire stimulus for the increased transcription we have observed. First, even though the increase in TCR-α gene expression is completely independent of protein synthesis, as has been shown for all other AP-1-binding PMA-inducible genes (48–50), the increase in TCR-β is not. Second, $D_\beta J_\beta C_\beta$ transcripts are also inducible in the absence of new protein synthesis. Although it is possible that cryptic AP-1 sites exist 5′ of the D region, the fact that $V_\beta D_\beta J_\beta C_\beta$ transcripts are also partly inducible in the absence of protein synthesis suggests that both $V_\beta D_\beta J_\beta C_\beta$ and $D_\beta J_\beta C_\beta$ transcripts may share common regulatory elements in the $D_\beta J_\beta C_\beta$ region. It is possible that the increased transcription and mRNA expression of the TCR genes results in part from a cellular response to the rapid decrease in the surface expression of the TCR/CD3 complex induced by PMA. The decrease in TCR density on T cell clones stimulated with PMA has been shown to be associated with a transient state of unresponsiveness to Ag (20, 21). Thus, protein kinase C activation may play an important role in the control of uninhibited proliferation in T cells. The decreased transcription and mRNA expression of the c-*myc* gene observed after PMA stimulation of Jurkat cells supports this hypothesis. c-*myc* expression has been shown to correlate with the proliferative capacity of lymphoid cells.

Recently, Shackelford et al. (51) reported that PMA will induce high levels of TCR-α mRNA in clones of the CCRF CEM T cell tumor line, which, in the uninduced state, expresses no TCR-α mRNA. There are, however, several differences in the system used in this study as compared with ours. First, the effect on TCR-β expression was variable in different clones. The PMA induction of TCR-α is accompanied by the appearance of an α-β heterodimer associated with CD3 on the surface of these cells, contrary to results with other tumor cell lines (Jurkat, HUT 78, HPB-ALL), T cell hybridomas, T cell clones as

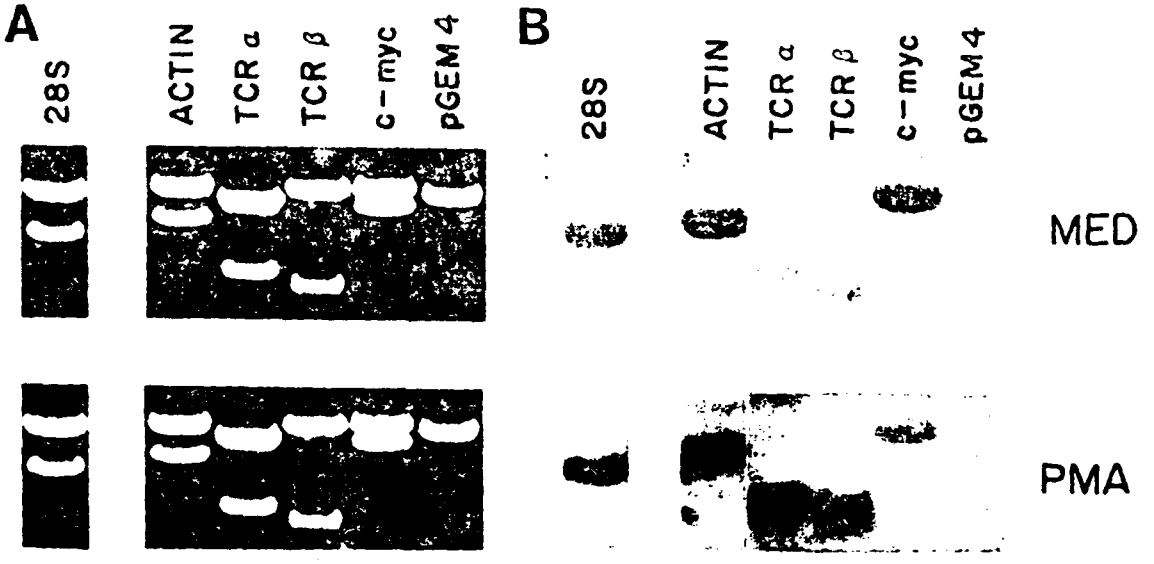

Figure 4. Run-on transcription assays of resting and PMA-activated Jurkat I cells. *A*, 5 μg of cDNA plasmids of the 28S gene, actin, TCR-α, TCR-β, c-*myc*, and pGEM4 genes were digested with the appropriate restriction endonucleases to release insert from vector. After digestion, the DNA was separated on a 1% agarose gel, stained with ethidium bromide, and transferred to nitrocellulose as described (42). *B*, Radiolabeled run-on transcription products from Jurkat cells incubated for 12 h in medium (*MED*) alone or with 10 ng/ml PMA were hybridized to Southern blots.

well as normal peripheral and thymic T cells (17, 20, 21). A possible interpretation of these results is that PMA induces a further state of differentiation in the CCRF CEM cells, as it has been shown that TCR-α gene expression occurs later in development than TCR-β and CD3 expression (52, 53). Thus, the results could reflect that a different cell line with different characteristics was used. It has been reported that PMA treatment of murine thymocytes also leads to increased TCR-α and -β mRNA levels (54). However, the mechanism behind this increase was not studied.

Our data indicate that the expression of the TCR-α and -β genes may be differentially regulated. As mentioned above, the induction of the TCR-α gene is independent of new protein synthesis. However, the induction of the TCR-β 1.3-kb $V_\beta D_\beta J_\beta C_\beta$ transcript, but not that of the 1.0-kb $D_\beta J_\beta C_\beta$ transcript, is partially dependent on new protein synthesis. More interestingly, the fact that the 1.0-kb $D_\beta J_\beta C_\beta$ transcript is inducible indicates the presence of a regulatory element independent of classical transcriptional elements present upstream of the V region gene segments. By analogy with Ig genes, this regulatory element could be an enhancer (55, 56). Recently, an enhancer element has been located in the J-C intron of the TCR-α gene (46); however, no similar reports exist for the TCR-β gene. Our study suggests a similar search should be conducted in the $D_\beta J_\beta C_\beta$ region for evidence of an enhancer element.

The fact that the surface expression of the TCR/CD3 complex diminishes with PMA treatment raises the question of how this might occur. Conflicting results appear in the literature. Reinherz et al. (19) have reported that the TCR/CD3 complex is shed after stimulation, whereas Krangel (23) showed that the complex is internalized. Our data demonstrate that the PMA-induced depression of the surface expression of the TCR/CD3 complex is not the result of a decrease in the transcription or mRNA expression of the TCR-α or TCR-β genes. In contrast, both the transcription and steady state mRNA levels of the TCR-α and TCR-β genes are increased 5- to 10-fold within 6 to 12 h after PMA treatment of Jurkat cells.

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